Polypeptide growth factors in gastroenteropancreatic neuroendocrine tumours

Czynniki wzrostu w guzach neuroendokrynnych przewodu pokarmowego

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Abstract

Polypeptide growth factors form a potent class of extracellular signal molecules in the regulation of cellular differentiation and proliferation. Disturbances in the expression of growth factors influence the normal pathway of differentiation and lead to cellular transformation and tumour progression. Contemporary medical studies report that various growth factors such as those for platelet-derived growth factor, vascular endothelial growth factor, epidermal growth factor, hepatocyte growth factor and insulin-like growth factor are expressed in gastroenteropancreatic neuroendocrine tumours (GEP/NET). Polypeptide growth factors have great significance in the growth, progression and development of metastases by various tumours. We describe the role of growth factors in GEP/NET on the basis of the available reports of medical research.

Key words: gastroenteropancreatic neuroendocrine tumours, growth factors, insulin-like growth factor, platelet-derived growth factor, vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor

Polypeptide growth factors form a potent class of extracellular signal molecules in the regulation of cellular differentiation and proliferation. Disturbances in the expression of growth factors influence the normal pathway of differentiation and lead to cellular transformation and tumour progression. Contemporary medical studies report that various growth factors such as those for platelet-derived growth factor, vascular endothelial growth factor, epidermal growth factor, hepatocyte growth factor and insulin-like growth factor are expressed in gastroenteropancreatic neuroendocrine tumours (GEP/NET). Polypeptide growth factors have great significance in the growth, progression and development of metastases by various tumours. We describe the role of growth factors in GEP/NET on the basis of the available reports of medical research.

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Introduction

Gastroenteropancreatic neuroendocrine tumours (GEP/NET) are generally considered to be slow-growing neo-

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plasms. However, in a significant subset aggressive growth occurs, resulting in decreased survival [1–3]. The aberrant expression of growth factors and/or aberrant responses to growth factors may circumvent the normal pathway of differentiation, leading to cellular transformation, tumour progression and maintenance of the transformed phenotype [4, 5]. The most common malignant symptomatic pancreatic endocrine tumour (PET) [6, 7] is a gastrinoma which, in 25% of patients, has an aggressive growth pattern, leads to the development of liver metastases and results in a 10-year-survival in 30%
of patients [8]. At present the factors responsible for these variable growth patterns in different PET as well as in gastrinomas are largely unknown. This situation exists because the molecular pathogenesis of NET has not been sufficiently investigated [9]. Recent studies report that various growth factors are expressed in gastroenteropancreatic neuroendocrine tumors (GEP/NET) (Table I) and play an important role in the growth, progression and development of metastases of various tumours [9–12]. These growth factors include fibroblast growth factors (αFGF, βFGF), transforming growth factors (TGFα, TGFβ), an epidermal growth factor (EGF), platelet-derived growth factors (PDGF), insulin-like growth factors (IGF1, IGF2) hepatocyte growth factor and interleukins (IL-1, IL-2).

Insulin-like growth factor 1 (IGF-1)

IGF-1 is a 70-amino-acid anabolic hormone. In normal conditions IGF-1 is produced by growth hormone (GH) in the liver [13]. Insulin-like growth factor receptor (IGF-1R) is a member of the tyrosine kinase (TK) receptor super-family with a 70% homology to the insulin receptor [11]. IGF-1R activation can induce numerous cellular effects, including differentiation, transformation and prevention of apoptosis. The activation of IGF-1R increases tumour growth and up-regulates vascular endothelial growth factor expression, promoting tumour invasion [14, 15]. Activation of IGF-1R causes activation of at least two signal cascades. The first cascade promotes the survival of cells by the sequential passing of information by phosphatidylinositol kinase 3 (PI3K), protein kinase B (PKB), GSFβ3, β-katenin and the transcriptional activator regulated by the Myc-TCT 4 protein. In the cells of a pancreatic tumour the activation of PKB can cause an up-regulation of expression of IGF-1R and positive feedback, which extends the survival of cells. In contrast, the second cascade (the cascade of Ras-Raf-MAPK) promotes cellular proliferation. Therefore different cascades activated by IGF-1R in different cellular arrangements can be partially determined by differences in the mode of activating them (Fig. 1). A high concentration of IGF-1 is recognised as a risk factor for the appearance of malignant tumours in the prostatic gland, breast and colon [13], but its expression pattern in the functionally and biologically heterogeneous human GEP/NET should be thoroughly elucidated [16]. Currently there are some reports of IGF-1 and/or IGF-1R as present in some NET and these are associated with an advanced tumour stage, increased tumour size, proliferative activity, recurrence or metastases and a poor prognosis/survival [17–21]. In isolated NET IGF-1 can stimulate tumour growth [22]. Other studies have reported no association between IGF-1/IGF-1R and tumour stage, size or survival [17, 18, 21, 23, 24]. In two studies involving different PET [9, 16, 25] and three studies involving GEP/NET [9, 16, 22, 25] the presence or absence of IGF-1 and/or IGF-1R did not correlate with tumour aggressiveness. However, no quantitative comparisons were performed in these studies [9, 16, 22, 25]. Increased IGF-1R mRNA expression in gastrinoma correlated significantly with increased tumour growth, aggressive disease and increased tumour extent, as, to a lesser degree, did IGF-1 expression.

Furukawa et al. [26] reported that both IGF-1 and IGF-1R mRNA expression levels are related to gastrinoma aggressiveness and that IGF-1R levels are predictive of disease-free survival, which could have clinical significance. The assessment of IGF-1R mRNA levels in the gastrinoma may allow stratification of patients to different risk levels, which could be used to determine risk and allow identification of patients requiring more careful follow-up. However, in the light of the increased development of possible therapeutic strategies directed against IGF-1R [10] and the effects of such drugs as somatostatin analogues in decreasing IGF-1 secretion, the possible involvement of IGF-1R in the molecular pathogenesis of these tumours, together with the link between its expression and tumour aggressiveness, raises the possibility that an approach directed against

<table>
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<th>Growth factor</th>
<th>Fore-gut NET</th>
<th>Midgut carcinoid</th>
<th>Hindgut NET</th>
<th>Gastrinoma</th>
<th>Insulinoma</th>
<th>Functionally inactive tumours in GEP/NET</th>
<th>Well-differentiated neuroendocrine tumors</th>
<th>PET</th>
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<tbody>
<tr>
<td>IGF-1</td>
<td>+ [44, 77]</td>
<td>+ [27, 50]</td>
<td>+ [30]</td>
<td>+ [30]</td>
<td></td>
<td>+ [30]</td>
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<td>VEGF</td>
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<td>EGF and HGF</td>
<td>+ [69]</td>
<td></td>
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<td>+ [47]</td>
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<tr>
<td>TGFα</td>
<td>+ [47]</td>
<td>+ [46, 47]</td>
<td>+ [27, 47]</td>
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IGF-1R could have therapeutic value in treatment of the tumours. In 2004 Van Gompel Chen [27] described the activation of a raf-1/MEK1 pathway which reversed the effect of IGF-1 treatment by the depletion of intracellular chromogranin A (CgA). The induction of the raf-1/MEK1 pathway blocks IGF-1-mediated intracellular neuroendocrine hormone regulation. Therefore raf-1/MEK1 activation may be a viable method for blocking IGF-1-mediated cellular effects and serve as a therapeutic target in gastrointestinal carcinoid tumours.

Von Wichert et al. [28] first presented the Ras/PI3K/AKT/Rac/NFκB/cyclin D1 signalling cascade. Constitutive expression of cyclin D1 is a frequent abnormality in human cancer and sustains the transformed phenotype. They previously demonstrated that cyclin D1 is constitutively expressed in human BON NET cells as a result of an autocrine IGF-1 loop. Their data provide the first comprehensive map of the signalling events elicited by endogenously released IGF-1 leading to constitutive cyclin D1 expression in human NET.

Wulbrand et al. [16] reported a study of IGF system components, including insulin-like growth factor binding proteins (IGFBPs), in the “European Journal of Clinical Investigation” in 2000. They showed differences in the expression patterns of the IGF system components in NET subtypes, which suggest pathways in tumour growth control that are differentiated according to tumour type by means of IGF system components [16]. IGFBPs are important in the carcinogenesis of several tumours, but their expression pattern in the functionally and biologically heterogeneous human GEP/NET has not been adequately identified [16]. There are several IGFBPs by which the total serum concentration of IGF-1 is maintained at a level 1000 times higher than the concentration of free insulin. Synthesis of IGFBPs, like that of IGF, depends on GH; both IGF-1 and GH induce the expression of IGFBPs, while insulin reduces it. By reducing the biological accessibility of IGF-1, IGFBP can modify free GH activity. The isoform of IGFBP present in blood serum in the largest quantity is IGFBP3.

Wulbrand et al. [16] analysed 37 tumour samples (9 gastrinomas, 10 insulinomas, 9 tumours associated with carcinoid syndrome and 9 functionally inactive tumours), in all of which IGFBP-2 was found, while IGFBP-1 was expressed only at a low frequency (10–22%) among the four tumour types. Because expression of IGFBP-2 correlates with the proliferation of some tumour cell lines and has been associated with an increased malignancy of certain tumours [29–31], IGFBP-2 could facilitate the autocrine action of IGF-1 and thereby increase its half-life [32].

Another study of IGFBP was published in “Clinical Cancer Research” in 2004. In this Donna E. Hansel [33] described the role of IGFBP3 and MET proto-oncogene

**Figure 1.** Different cascades activating growth factors in different cellular arrangements. Kinase tyrosine receptors activate Ras-Raf-MAP (serine-treonine kinases), PI3K phosphatidylinositol kinase 3, protein kinase C (PKC) and calcium.
with metastatic ability in well-differentiated pancreatic endocrine neoplasms. IGFBP3 functions as a carrier molecule for both IGF-1 and IGF-2 in the circulation [34, 35]. IGFBP3 mediates both pro- and anti-proliferative effects on various cell types [35]. Increased serum levels of IGFBP3 have been associated with the progression of breast cancer in several studies [36, 37]. Overexpression of IGFBP3 in non-metastatic pancreatic endocrine neoplasms as opposed to normal human islet cells has previously been identified [38]. Analysis of IGFBP3 expression in metastatic compared with non-metastatic pancreatic endocrine neoplasms identified IGFBP3 expression in 42% of non-metastatic pancreatic endocrine neoplasms and 80% of metastatic primary pancreatic endocrine neoplasms. In addition, IGFBP3 expression was identified in 86% and 100% of lymph node and liver metastases respectively.

MET functions as a transmembrane receptor of TK that is activated by hepatocyte growth factor/scatter factor [39]. Inappropriate expression of MET has been documented in the majority of solid tumour types and often appears to correlate with a worsened prognosis [40]. MET signalling results in disruption of cell-to-cell adhesion, branching morphogenesis and invasive and metastatic behaviour by a large array of neoplasms [41]. The expression of MET has been identified in 17% of non-metastatic pancreatic endocrine neoplasms compared with 33% of primary pancreatic endocrine neoplasms demonstrating concurrent metastases. MET expression appeared most prevalently in lymph node (57%) and liver (56%) metastases. Like IGFBP3, MET expression may also demonstrate a continuum of expression with neoplastic progression [33].

Another problem in medical studies concerns the autocrine action of IGF-1/IGFR [32, 42]. Exogenously added IGF-1 induces a marked increase in the secretion of CgA, a marker protein for neuroendocrine secretion, by a process that is largely dependent on PI3-kinase activity. In addition, immunoneutralisation of endogenously released IGF-1 markedly reduces the basic chromogranin secretion level. The constitutive activation of certain kinases under serum-free conditions is increasingly appreciated as a mechanism leading to the autonomous growth of tumour cells in culture. It has been suggested that the PI3-kinase-phosphorylated products of phosphatidylinositol play a role in the regulation of membrane trafficking along secretory pathways, for example in chromaffin cells [43]. Therefore by targeting either PI3-kinase or endogenously released IGF-1, both autocrine and neuroendocrine secretory pathways can be substantially blocked in BON cells. Targeting IGF-1 or the IGF-1 receptor TK may constitute a novel therapeutic strategy for patients suffering from NET. Endogenously released IGF-1 is found to be largely responsible for the autonomous growth of BON cells in a serum-free medium and for the constitutive expression of cyclin D1 in these cells. In conclusion, IGF-1 is a major autocrine regulator of neuroendocrine secretion and the growth of human BON NET cells [42].

The epidermal growth factor family of polypeptide growth factors

Transforming growth factor α (TGFα)

Transforming growth factor α is one of the growth factors that are similar to epidermal growth factors (EGF) [13]. It is a 50-amino-acid polypeptide that binds to the epidermal growth factor receptor (EGFR) and stimulates cell growth. It has been suggested that enhanced production of TGFα and EGFR by tumour cells promote tumour-cell growth by autocrine mechanisms [44]. Krishnamurthy and Dayal [45] analysed the expression of TGFα and EGFR in mid-gut, fore-gut and hind-gut NET in a study in 1997. They reported that although TGFα is expressed by a high proportion of these tumours, the absence of its intact EGFR molecule on the tumour cells renders it functionally ineffective as a growth factor. Thus, in contrast to its influence on tumours of the gastrointestinal tract, TGFα appears to play no role in the growth and progression of mid-gut, fore-gut and hind-gut NET, which perhaps explains the indolent behaviour and slow biological progression of GEP/NET.

In another paper Nillson et al. [44] also evaluated expression of TGFα and EGFR in phaeochromocytomas and medullary thyroid carcinomas. TGFα expression was demonstrated in biopsies of all the tumours examined (n = 30) and EGFR receptors in the majority of tumours by Northern analysis and/or immunocytochemistry. Expression of TGFα and EGFR receptors was also demonstrated in primary cultures of tumour cells. The amount of secreted TGFα could be suppressed by octreotide treatment in individual tumours. The growth-stimulatory effect of TGFα could be partially blocked by the use of neutralising anti-EGF receptor monoclonal antibodies (Mabs). In conclusion, several human NET express both TGF-α and EGFR in vivo and in vitro, suggesting that TGFα may regulate tumour-cell growth by autocrine mechanisms.

Epidermal growth factor (EGF)

Epidermal growth factor is one of the smallest of the growth factors. It is a 33-amino-acid polypeptide splintered off a large precursor binding to the membrane [13]. EGF, like all growth factors, binds to specific high-affinity, low-capacity receptors on the surface of responsive cells. Intrinsically to the EGF receptor is TK activity, which is activated in response to EGF binding. The ki-
Platelet-derived Growth Factor (PDGF)

Platelet-derived growth factor is composed of two distinct polypeptide chains, A and B, which form homodimers (AA or BB) or heterodimers (AB). The c-Sis proto-oncogene has been shown to be homologous to the PDGF A chain. Only the dimeric forms of PDGF interact with the PDGF receptor. Two distinct classes of PDGF receptor have been cloned, one specific for AA homodimers and another that binds BB and AB type dimers. Like the EGF receptor, the PDGF receptors have intrinsic TK activity. Following autophosphorylation of the PDGF receptor, numerous signal-transducing proteins associate with the receptor and are subsequently tyrosine phosphorylated. Proliferative responses to PDGF action are exerted on many mesenchymal cell types. Other growth-related responses to PDGF include cytoskeletal rearrangement and increased polyphosphoinositol turnover. Again, like EGF, PDGF induces the expression of a number of nuclear localized proto-oncogenes, such as Fos, Myc and Jun. The primary effects of TGF-β are due to the induction, by TGF-β, of PDGF expression [46].

Chaudhry et al. in their 1993 study [62] reported that multiple peptide growth factors, PDGF, TGF-β, and βFGF are expressed by GEP/NET. PDGF was expressed on tumour cells and stroma in 70% of the tissues examined. PDGF alpha-receptor was seen on clusters of tumour cells and occasionally on adjacent stroma, whereas PDGF beta-receptor was seen only in the stroma. Their data suggest that PDGF may be involved in the autocrine stimulation of tumour cells and stimulation of stromal cell growth through a paracrine and possibly an autocrine mechanism.

Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (also known as VEGF-A, but commonly referred to simply as VEGF) stimulates vascular endothelial cell growth, survival, and proliferation. It plays a significant role in the development of new blood vessels (angiogenesis) and the survival of immature blood vessels (vascular maintenance). VEGF binds to and activates two related receptors found on the endothelial cell membrane. These are known as VEGF receptor-1 (VEGFR-1 or flt-1) and VEGFR-2 (KDR or flk-1) and are expressed by endothelial cells within the blood vessel wall. VEGF also interacts with the structurally distinct receptors neuropilin (NP)-1 and NP-2 (which are normally expressed on endothelial cells and enhance the mitogenic effects of VEGFR-2). The binding of VEGF to these receptors initiates a signalling cascade that affects the survival, proliferation, and migration of endothelial cells, ultimately leading to angiogenesis [63, 64]. VEGF expression/overexpression has been shown to be a key mediator of angiogenesis across multiple tumour types, including colorectal, lung, breast and other cancers. Across each of these cancers a number of interrelated signals and processes have been identified as leading to the production of VEGF and, ultimately, the neovascularisation of a tumour [65].

In 2003 la Rosa et al. [66] reported expression of VEGF and its receptors did not correlate with micro-
vessel density or malignancy. These results suggest that in normal tissues endothelial functions may be regulated by VEGF produced by some endocrine cells and that a VEGF/VEGFR binding mechanism may be involved in tumourigenesis but not in tumour progression and aggressiveness.

In another paper Terris [67] demonstrated that neuroendocrine cells are a major source of VEGF, particularly in carcinoids. This finding suggests that the presence of VEGF may be required to maintain the differentiated state of capillary vessels in these hypervascular tumours. Such secretion, in conjunction with the other growth factors synthesised by these NET, may have an important role in tumour growth. No correlation between VEGF expression and tumour stage was found.

Neuropilin-2 (NP-2)

Neuropilin-2 (NP-2) is a cell surface transmembrane protein originally characterised as a receptor for type 3 semaphorins and, more recently, for a number of vascular endothelial growth factor (VEGF) isoforms [68].

Cohen et al. [68] analysed the expression of NP-2 in pancreatic islet cells and PET as a novel marker. NP-2 expression has recently been localised to a subset of neuroendocrine cells in the gastrointestinal tract. NP-2 expression was not detected in neuroendocrine cells outside the gastrointestinal system or in their corresponding neoplasms, except for focal staining in one bronchial carcinoid tumour. In conclusion, the vast majority of PET examined expressed NP-2, suggesting its utility as a diagnostic marker for these tumours. The function of NP-2 in islet cell biology or tumourigenesis remains to be elucidated.

Fibroblast Growth Factors (FGFs)

Endocrine tumours (ETs) of the digestive system produce several growth factors, including acidic and basic (αFGF and βFGF respectively), which are thought to be involved in the growth of tumour cells and in the proliferation of tumour stromal cells.

La Rosa et al. [69] described the immunohistochemical detection of FGF receptors in normal endocrine cells and related tumours of the digestive system. Enterochromaffin cell (EC) tumours, which were all positive for αFGF, were found to express at least three different fibroblast growth factor receptors (FGFRs). FGFRs were also localised in the stromal cells of all the tumours examined. The tumour stroma was more abundant in EC cell tumours than in other types of neoplasm. The results suggest that αFGF-FGFR interaction may be involved in the modulation of normal endocrine cell functions and in the regulation of tumour growth and stromal proliferation of EC cell tumours.

Treatment of GEP/NET

The treatment of choice for GEP/NET is surgery. Surgery should be considered in cases with liver metastases and potentially resectable tumour. For patients who are not fit for surgery the aim of treatment is to improve and maintain an optimal quality of life. The choice of treatment depends on the symptoms, stage of disease, degree of radionuclide uptake and histological features of the tumour. Treatment choices for non-resectable disease include somatostatin analogues, biotherapy, chemotherapy, radionuclides and ablation therapies [70]. The anti-neoplastic therapy of advanced NET is still unsatisfactory and innovative therapeutic approaches are needed [71].

At present intensive research is being conducted on new drugs, including inhibitors of growth factors. This therapy could turn out to be indispensable in the future because of the great role played by growth factors in the development and pathogenesis of GEP/NET. Apart from the IGF-1R TK inhibitor described, different inhibitors of growth factors are enumerated in the literature, although the investigations do not concern GEP-NET. The medications include:

— AEE788, a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor TK inhibitor with an anti-tumour and anti-angiogenic action (cell lung cancer, glioblastomas, and breast tumours) [72];

— SU6668, a potent anti-angiogenic and anti-tumour agent that induces regression of established tumours (glioma and melanoma of lung, colon, ovarian, and epidermoid origin) [71];

— SU11248, a novel TK inhibitor targeting VEGF and PDGF receptors [73].

The inhibition of the IGF/IGF-receptor system may offer possibilities for novel targeted treatment strategies of NET because these frequently express insulin-like growth factors and their receptors, which are known to promote survival, oncogenic transformation, tumour growth and spreading [74].

Hopfner et al. [74] described the anti-neoplastic effects of the inhibition of IGF-1R signalling in NET cells by the novel IGF-1R-TK inhibitor NVP-AEW541, whose anti-neoplastic potency has not yet been tested in NET disease. Apoptosis was characterised by activation of the apoptotic key enzyme, caspase-3, as well as by detection of changes in the expression of the pro- and anti-apoptotic proteins, BAX and Bcl-2, after NVP-AEW541 treatment. The cell cycle was arrested at the G1/S checkpoint. The anti-neoplastic effects of NVP-AEW541 involved the inactivation of ERK1/2. The induction of immediate cytotoxicity did not account for the anti-neoplastic effects of NVP-AEW541, as shown
by measurement of lactate dehydrogenase release. Moreover, additive anti-neoplastic effects were observed when NVP-AEW541 was combined with cytostatics such as doxorubicin or the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, fluvastatin. This is the first report on the induction of apoptosis and cell cycle arrest by the IGF-1/IR-TK inhibitor NVP-AEW541 in NET cells. The inhibition of the IGF-1/IGF-1R system appears to be a promising novel approach for future treatment strategies of GEP/NET.

There is a need for more extensive research into tumour biology, including that concerned with the roles of growth factors. A better understanding of the molecular biology of these tumours may lead to better clinical models for predicting outcome and developing novel treatment strategies for this relatively rare but complex disease.

References


